Output 2: Improved large-seeded Andean bean germplasm with less dependence on inputs

Activity 2.1 Developing germplasm resistant to diseases

Highlights:

- Genetic improvement efforts to incorporate BCMV resistance in highland common bean cultivars are under way.

- Six genotypes with high levels of resistance to race 651 and 653 were identified.

- Red-mottled beans for the Andean and Caribbean regions were improved for disease resistance by pyramiding sources of resistance to ALS and by using marker-assisted selection to incorporate BGMV-resistance genes. *Empoasca* and bruchid resistances were combined into lines containing virus resistance.

- Cream-mottled beans were improved for yield and quality by crossing with Colombian Cargamanto beans and incorporating BCMV-resistance and bush type architecture.

- A new nursery of Andean genotypes (IBN 2000) was tested for ALS and CBB resistance and distributed to collaborators.

2.1.1 Breeding for resistance to bean common mosaic virus

Rationale: Bean common mosaic virus resistance has been a *sine qua non* requirement for most breeding lines generated by the CIAT Bean Project in collaboration with NARIs. The relatively low incidence of BCMV in the Andean highlands observed in the past somehow relegated this task to second place in past years. However, the increasing frequency of the El Niño phenomenon has created suitable climatic conditions (warming) in the Andean highlands for the aphid vectors of this virus. Consequently, BCMV epidemics have occurred in the Andean highlands, and particularly in the region of Rionegro, Antioquia, Colombia, where the predominant, high-value cultivar "Cargamanto" has suffered severe BCMV attacks in past years. A project coordinated by Ms. Gloria Esperanza Santana of the Corporación Colombiana de Investigación Agropecuaria (CORPOICA)-Rionegro, with the collaboration of CIAT, is trying to introduce resistance to BCMV in this climbing, common bean cultivar.

Materials and methods: 50 different genotypes including climbing cultivars, germplasm bank accessions, "Cargamanto" varieties, breeding lines, and some sources of BCMV resistance, were screened for their reaction to a strain of bean common mosaic necrosis virus (BCMNV), which also helps identify bean genotypes with resistance to BCMV. Farmers in the region currently grow nine cultivars and three improved cultivars (ICA Viboral, ICA-Llanogrande, and Frijolica L.S. 3.3).

Results: Eight breeding lines from Cargamanto x G 17668 crosses made at CIAT were resistant to BCMV. The remaining materials were either segregating or susceptible to BCMV. Nine germplasm bank accessions maintained by CORPOICA were also susceptible to BCMV. Of three CIAT breeding lines selected for their resistance to anthracnose, another prevalent disease of climbing beans in Rionegro, only one was resistant to BCMV. Another set of nine bush beans possessing anthracnose resistance was also evaluated and shown to include two genotypes that were homozygous resistant. Finally, three lines selected as sources of the BCMV-resistance recessive gene *bc 3* were confirmed as resistant to this virus and thus are potential sources of virus resistance to improve Cargamanto for this character, without the undesirable genetic linkage problems associated with the dominant *I* gene.

Conclusions: Crosses with parental materials possessing the recessive *bc 3* gene must be initiated in order to preserve the commercial red color of the characteristic Cargamanto grain type. Other sources of the *I* gene from the Andean gene pool have also been recommended to avoid the linkage problems associated with the incorporation of monogenic dominant resistance, but this hypothesis has not been thoroughly tested.

Contributors: FJ Morales, M Castaño, CJ Alvarez

2.1.2 Identifying genotypes with resistance to anthracnose

Rationale: Identification of resistance to major diseases is a continuous activity. Anthracnose is a major limitation of production in the highlands of Colombia, especially in the Rionegro area of Antioquia. Last year, we reported on a project to identify sources of anthracnose resistance to race 651 and 653. This study is a follow up to last year's evaluations and includes a wide genetic base of bean germplasm.

Materials and methods: In collaboration with CORPOICA Rionegro, we screened 40 bean lines for their reaction to anthracnose races 651 and 653. Ten plants of each genotype were evaluated in each of the two repetitions. Inoculum production, inoculations, and evaluations were done according to standard protocols.

Results and discussion: The genotypes G 4, LAS 129, LAS 435, SUG 130, SEQ 1035 and PR 93201473 were resistant to both race 653 and 651 (Table 27). These genotypes form a highly interesting group of materials that can either be deployed or used as sources of anthracnose resistance in breeding programs. Some genotypes (e.g., G 6, G 8, LAS 331, ICA Llanogrande, BRB 204, and FOT 54) had a resistant reaction with one race and an intermediate reaction with the other. Given that the inoculum concentration is usually high under controlled conditions, these genotypes might serve as good sources of resistance under field conditions, where inoculum loads are generally low.

Conclusion: Six genotypes (G 4, LAS 129, LAS 435, SUG 130, SEQ 1035, and PR 93201473) with high levels of resistance to race 653 and 651 were identified in this study. These can be used as parents in crosses, but they first must be evaluated under field conditions, to get a feeling of the activity of the resistance genes.

Table 27. Reaction of 36 advanced bean lines to race 651 and 653 of *Colletotrichum lindemuthianum.*

Entry	Disease rating[a]		Entry	Disease rating[a]	
	Race 653	Race 651		Race 653	Race 651
G 1	R	S	CC21148-218-3	S	S
G 3	I	I	CC21148R218-4	S	S
G 4	R	R	CC21148-218-5	S	S
G 5	I	I	CC211482-18-6	S	S
G 6	R	I	CC21148 R-5-1	S	S
G 7	R	S	CC21148-R-59-1	S	S
G 8	R	I	CC21148-67-3	S	S
G 9	R	S	BRB 151	R	S
G 11	S	S	BRB 203	R	S
LAS 106	I	I	BRB 204	I	R
LAS 129	R	R	SEQ 1035	R	R
LAS 331	I	R	SEQ 1040	S	R
LAS 384	R	S	FOT 28	R	S
LAS 435	R	R	FOT 29	R	S
LAS120	S	S	FOT 54	I	R
AND 1084	I	I	FOT 59	R	S
ICA Llanogrande	R	I	SUG 130	R	R
CC21148-4-1	S	S	SUG 143	R	S
FOT 49	I	R	PR 93201473	R	R
G 17668	S	S	PR 93201474	R	R

a. R = resistant, I = intermediate, and S = susceptible.

Contributors: G Mahuku, C Jara, G Castellanos; GE Santana (CORPOICA)

2.1.3 Pyramiding angular leaf spot resistance into Calima-type, red-mottled beans

Rationale: Angular leaf spot is a serious disease in humid low-and mid-elevation bean production areas. A fungal pathogen, *Phaeoisariopsis griseola*, which is well known for its pathogenic variability, is the cause of the disease. Resistance is often location-specific depending on the races prevalent in a given area. The disease reaction exhibited by leaves and pods from the same genotype can be different, suggesting that separate genes can control resistance in these organs. Therefore pyramiding various sources of resistance is important when trying to develop new varieties that will be field resistant to the widest array of races in many bean-growing regions. The objective of this research was to compare advanced lines, which were developed from multiple crosses that pyramid ALS-resistant parents together, for their level of field resistance to the disease.

Materials and methods: In Darien in 2000A, 834 Andean genotypes, representing the red-mottled, cream-mottled, large-red, and yellow-seeded bean classes, were grown in single 3-m row plots. The genotypes consisted mostly of $F_{5:6}$ lines from multiple crosses made in 1996-97, which were advanced through a gamete selection program over the course of 4 years. A smaller number of earlier generation F_3 bulks was also tested. The crop was fertilized with 100 kg ha^{-1} of superphosphate, but was not protected with fungicides. A natural epidemic of ALS occurred in the second half of the growing season. The lines were evaluated for ALS resistance at 40 days after planting with the standard 1-9 scale for CIAT evaluations.

Results and discussion: The average level of resistance found in the advanced lines from a given pedigree was correlated with (1) whether the final parent was ALS resistant, and (2) how many parents in the multiple cross pedigree were ALS resistant (Table 28). The average ALS scores for the pedigrees ranged from 1.94 to 7.00. The most outstanding pedigrees included the parents AND 277, AND 279, Cornell 49-242, G 5686, and MAR 3, which are all well-known sources of ALS resistance. The genotypes CAL 143 and PVA 800A also appeared to be proportioning resistance to some of the crosses. Crosses with no resistant parents such as PVA 773 x (A 483 x G 6416) were highly susceptible. The advanced lines performed well compared to the susceptible red-mottled checks A 36, A 483, AFR 619, AND 1005, CAL 96, and ICA Tundama. The best red-mottled checks were AFR 612, AND 277, AND 279, POA 12, and PVA 800A.

Conclusions and future plans: Good levels of resistance to the prevalent ALS isolates existed in the red-mottled seed class parents and progeny we tested in Darien. The popularity of AFR 612 in Cauca may be partly because of its good ALS resistance. Several other parents were also successful in other parts of the world where ALS is a problem, so we are hopeful that the material developed here will be useful in other regions. POA 12 is being included in new crosses. The advanced lines from this trial were evaluated for commercial seed type by a farmer-led participatory research committee (a CIAL) from Restrepo, Valle that will be testing the lines for performance in their fields in 2000B. The parents of these populations were included in crosses made to study the inheritance of ALS resistance.

Contributor: MW Blair

Table 28. The angular leaf spot (ALS) scores of red-mottled advanced $F_{5:6}$ lines from different crosses of multiple ALS-resistant parents (indicated in bold).

Pedigree	No. lines	Ave. ALS
(DRK 138 x Pompadour J)F_1 x (**PVA 800A** x ((DOR 708 x G 1344)F_1 x (A 429 x A 193)F_1)F_1)F_1/	33	NA
ICA Quimbaya x A 483	18	NA
(MAM 48 x AFR 612)F_1 x (PI 150414 x **PVA 800A**)F_1/	1	NA
(San Cristobal 83 x ICA Quimbaya)F_1 x (**PVA 800A** x **AND 277**)F_1/	14	NA
PVA 800A x (Paragachi x ((MAM 49 X Bola 60 Dias)F_1 x (**PVA 800A** x Bayo MEX)F_1)F_1)F_1/-	23	NA
Paragachi x (Paragachi x((MAM 49 x Bola 60 Dias)F_1 x (**PVA 800A** x Bayo MEX)F_1)F_1)F_1/-	1	NA
ARA 18 x (I 414 x ((**PVA 800A** x Bayo MEX)F_1 x (CAP 4 x Wilkinson 2)F_1)F_1)F_1/-	1	NA
AND 1005 x ((Catrachita x Bola 60 Dias)F_1 x (MAM 13 x Montcalm)F_1)F_1/	33	4.24
A797 x ((**JaloEEP558** x (Indeterminate Jamaica Red x Wilkinson 2)F_1)F_1/	4	3.50
AND 279 x ((MAM 38 x CAL 143)F_1 x (**PVA 800A** x **AND 277**)F_1)F_1/	139	2.50
(**AND 279** x **PVA 800A**)F_1 x ((**Cornell 49-242** x **G 5686**)F_1 x (Montcalm x **CAL 143**)F_1)F_1/	18	1.94
(Cardinal x **PVA 800A**)F_1 x ((Catrachita x PVA 773)F_1 x (**PVA 800A** x **AND 277**)F_1)F_1/	6	2.00
PVA 773 x ((MAM 48 x AFR 612)F_1 x (PI 150414 x **PVA 800A**)F_1)F_1/	96	2.86
(PVA 773 x ICA Tundama)F_1 x (**PVA 800A** x ((XAN 309 x A 193)F_1 x (**MAR 3** x G 5653)F_1)F_1)F_1/	109	2.51
(**PVA 800A** x **AND 277**)F_1 x (**PVA 800A** x ((XAN 309 x A 193)F_1 x (**MAR 3** x G 5653)F_1)F_1)F_1/	2	2.00
PVA 800A x ((San Cristobal 83 x ICA Quimbaya)F_1 x (**PVA 800A** x **AND 277**)F_1)F_1/	61	2.15
I414 x ((**PVA 800A** x G 5896) x (CAP 4 x Wilkinson 2))	12	4.42
PVA 1441 x ((MAM 49 x Bola 60 Dias) x (**PVA 800A** x G 5896))	4	4.50
PVA 773 x (A 483 x G 6416)	6	7.00
PVA7 73 x ((AND 1005 x (Catrachita x Bola 60 Dias)) x (MAM 13 x G 6416))	4	3.75
(**PVA 800A** x PVA 1441) x ((MAM 49 x Bola 60 Dias) x (**PVA 800A** x G 5896))	8	3.13
Total	594	3.32

2.1.4 Marker-assisted selection of bean golden mosaic virus-resistant red-mottled beans

Rationale: Bean golden mosaic virus is the most serious viral disease of beans in lowland tropical Latin America. A bipartite geminivirus, which is transmitted by the whitefly, *Bemisia tabaci*, causes the disease. Red-mottled beans are one of the few Andean types that are grown in low- and mid-altitude areas of the region where the vector and virus are present. The virus is a well-established endemic problem in red-mottled beans grown in the Caribbean (Haiti and the Dominican Republic) and has the potential to be a serious threat to some production areas in South America (Colombia, Ecuador, and especially lowland Bolivia). Other diseases that often occur when BGMV is present are CBB, rust, and ALS. The whitefly vector often occurs in conjunction with heavy infestations of leafhoppers. Therefore, it is important to pyramid genes for resistance to all these pests and pathogens together with BGMV resistance into the same genotypes. The objective of this work was to deploy BGMV resistance genes into new red-mottled genotypes through MAS of advanced lines in later generations. Because BGMV cannot be field-screened at CIAT and the virus is difficult to inoculate artificially, MAS is a good substitute for phenotypic selection.

Materials and methods: Two molecular markers were used to select for BGMV resistance in red-mottled beans. The SCAR marker, DOR 21, is closely linked to the major recessive resistance gene, *bgm-1*, while a second SCAR marker, W012, is associated with a QTL for BGMV resistance found on chromosome B04. A third SCAR marker, SU 91, was used to select for a major QTL for CBB resistance in a subset of the segregating lines.

Results and discussion: In the first set of trials, BGMV resistance was incorporated into advanced, red-mottled breeding lines through gamete and pedigree selection. Of 334 single plant selections made in the F_6 generation, 40 of the resulting $F_{6:7}$ lines were positive for the *bgm-1* marker. The positive lines were derived from two crosses,
(1) PVA 773 x ((PVA 800A x DOR 482)F_1 x (Belmineb RMR-3 x Montcalm)F_1)F_1, where the source of BGMV resistance was DOR 482, and (2) Calima x (MAM 48 x A 483)F_1)F_1 x (PVA 800A x ((EMP 376 x A 193)F_1 x (NW 63 x A 429), where A 429 was the source of BGMV resistance. The new advanced lines were used in crosses with larger-seeded, determinate red-mottled beans. Several red-mottled lines from the University of Puerto Rico, with resistance derived from DOR 482, were also used as donor parents. In addition, the segregating F_1 plants from the multiple cross, PVA 800A x ((A 429 x XAN 309) x (RAZ 44 x Royal Red)), were screened for the presence of arcelin and the *bgm-1* marker before being crossed to a group of Andean genotypes. These included AFR 619, Calima, CAL 96, CAL 143, CIAS 95, JB 178, PC 50, Quimbaya, Saladín 97, and Velasco Largo (Table 29). Twenty-two cross combinations were made, resulting in a total of 552 selected F_1 plants. The progeny of these crosses are being advanced to homozygosity when they will be screened again to make sure they contain the arcelin and *bgm-1* genes.

Table 29. Crosses with bean golden mosaic virus-positive plants from a multiple cross F_1 of PVA 800A x ((A 429 x XAN 309) x (RAZ 44 x Royal Red)).

Final parent	Color	Cross	Gametes	Final parent	Color	Cross	Gametes
AFR 619	6M,G	3	54	JB178	7M,M	1	23
Calima	6M,G	1	9	PC50	7M,P	5	94
CAL 96	6M,G	2	52	Quimbaya	7,G	1	15
CAL 143	6M,G	3	94	Saladín 97	7M,P	1	122
CIAS 95	7M,M	1	20	Velasco Largo	5,G	3	58

In the second set of trials, a single backcross was used to transfer the *bgm-1* gene and CBB QTL into a Pompadour variety (PC 50 or G 18264 from the Dominican Republic). SAM 1, a line derived from the cross DOR 476 x SEL 1309 and positive for all three SCAR markers, was the donor parent for these crosses. Twenty-one individual plants were selected in the BC_1F_1 generation and advanced two generations by pedigree selection. Eight $BC_2F_{2:3}$ progeny per family were grown in the greenhouse for MAS. DNA was extracted from a balanced bulk of plant tissues from each family member and genotyped for each of the SCARs. Although eight families were positive for *bgm-1*, four for the CBB QTL, and one for both markers, none of the families contained all the genes in a phenotype resembling the recurrent parent PC 50 (Table 30). However, several families were phenotypically very similar to PC 50 and contained a single resistance gene. Therefore, families that were complementary for the markers that they contained were crossed to produce PC 50 like segregants with more than one resistance gene.

Table 30. $BC_1F_{2:3}$ families positive for bean golden mosaic virus resistance markers in PC 50 (G 18264).

Selection[a]	bgm-1 (DOR21)	CBB (SU91)
- 2W	+	+
- 4W	-	+
- 5W	+	-
- 6W	-	+
- 7W	+	-
-11W	+	+
-22W	+	-
-24W	+	-
-29W	+	-
-37W	+	-

a. Pedigree of selections = G 18264 x (G 18264 x SAM 1).

Conclusions and future plans: Here we show the application of MAS to determine which advanced $F_{6:7}$ lines carry a gene of interest. MAS has also been successfully applied to selection of segregating F_1 plants from multiple crosses in early generations. Once we have incorporated the *bgm-1* gene widely into red-mottled germplasm, we plan to use the W012 SCAR more intensively in MAS of BGMV resistance. We also plan to pyramid BGMV resistance into red-mottled populations with *Empoasca* resistance and arcelin-based resistance to bruchids (see Output 2.2). Although the emphasis has been on bush beans we also plan to add BGMV resistance to red-mottled climbing beans, which might be grown in the Caribbean.

Contributors: MW Blair, S Beebe

2.1.5 Development and yield-testing of bean common mosaic virus-resistant, Cargamanto-type, cream-mottled, bush and climbing beans

Rationale: Cream-mottled beans are popular in many areas of the world, including southern Africa, southern Europe, and the Middle East. The class includes both Cranberry and Sugar beans. In Colombia, a special type of cream-mottled beans known as Cargamanto is grown. These are larger than the other cream-mottled seed types and are renowned for their flavor and cooking characteristics. Most Cargamanto varieties are aggressive type IV climbing beans adapted to altitudes above 2000 m in the departments of Antioquia and Nariño. The objective of this study was to use a Cargamanto variety to improve the seed types of cream-mottled bush beans and to incorporate BCMV resistance into both bush and climbing Cargamanto-type beans.

Materials and methods: Triple crosses were made between BCMV resistant (*I* + *bc3* genes) Cranberry bush beans and Cargamanto-type climbing bean selections. The Cargamanto lines (SEL 1383, SEL 1384, SEL 1386, SEL 1389, and SEL 1390) were derived from a simple cross between the commercial Cargamanto variety, ICA Viboral (G 5702), and Cran 28, which is a source of *I* gene for BCMV resistance that is not associated with dark-red seed mottling. These advanced lines were crossed to the F_1s of eight simple crosses between BCMV resistant lines, BRB 151 and BRB 203, which contain the *bc3* gene, with the Cranberry lines, FOT 28, FOT 54, and SUG 30. The F_1 plants were individually harvested and their progeny planted in Darien. Single F_2 plant selections from this generation were divided into three groups: (1) late type IIIs, which were planted in Nariño, (2) early type IIIs, which were planted in Antioquia, and (3) bush beans, which were planted in Darien. In the $F_{2:3}$ generation, the bush bean selections were planted in a replicated yield trial in Palmira.

Results and discussion: One hundred and twenty nine type III selections (67 early and 62 late) were sent to Nariño and Antioquia. Most of these were not adapted to the higher elevations at these locations and only 20 lines were selected for a second planting. Meanwhile, 311 selections were made among the bush beans in Darien. These were taken to a replicated trial during the next season to determine if they would adapt to the higher temperatures in Palmira compared to Darien. A large number of superior genotypes were identified among the $F_{2:3}$ selections, many of which surpassed the 12 cream-mottled check varieties grown in the experiment (Table 31). Most importantly, many of the new genotypes yielded as well as the best check variety, COS 16, and had the large, round seed size and shape of Cargamanto beans. This is the grain quality that commands a premium price in the marketplace.

Conclusions and future plans: The selections made during this season will be tested for the presence of several diagnostic molecular markers for BCMV resistance. These results will be confirmed with viral inoculation if needed. The climbing bean lines selected at the University of Nariño and CORPOICA-La Selva (Antioquia) will be grown for the second time this year. The bush bean lines will be offered to collaborators in Colombia, Bolivia, and Iran for testing in these environments. The new lines compare favorably to the Cranberry lines developed at CIAT for adaptation to mid-elevation areas and warm growing environments such as Palmira. One of these lines, COS 16, was released as a variety in Bolivia. Bolivian farmer and producer associations hope to find Cargamanto beans that will be acceptable in the Colombian market. We plan to test the Cargamanto-derived lines in Bolivia and Colombia at low and mid elevation growing areas to see if they perform as well as COS 16. We hope that some of the new Cargamanto bush lines may provide high yield and excellent grain quality for Latin American producers and consumers of the cream-mottled seed class.

Table 31. Yield of Cargamanto-type bush beans compared to Cranberry and Sugar check varieties in a replicated trial in Palmira, 2000A.

	Size	Yield per plant (no. of pods per plant)	Yield per hectare (kg)
Best line	Small (12)	17.0	2877
	Medium (203)	25.0	3773
	Large (96)	19.5	2592
Best 10 lines	Small (12)	11.1	1529
	Medium (203)	20.6	2851
	Large (96)	17.5	2309
Checks	BRB 151	14.0	2151
	BRB 203	12.0	1893
	COS 16	18.3	2577
	FOT 28	11.3	1536
	FOT 54	12.8	1993
	SEQ 1027	14.2	1846
	SEQ 1040	12.3	1642
	SUG 46	12.3	1706
	SUG 47	10.0	1564
	SUG 130	10.2	1774
	SUG 131	10.5	1188
	SUG 137	14.0	1969
CV		34.1	38.6

Contributors: MW Blair, S Beebe

2.1.6 Crosses for anthracnose-resistant climbing and bush type popping beans (ñuñas)

Rationale: Ñuñas or popping beans are a special class of common bean whose seed will burst open and expand to double their size when toasted, fried, or microwaved. They are a traditional crop of the Andean highlands of South America. Almost all varieties of ñuñas are climbing beans, which have a good yield potential, but require a long growing season. As a result of the extended time that ñuñas are in the field, they are exposed to a range of diseases, such as anthracnose, *Ascochyta*, halo blight, and rust. The objective of this research is to improve popping beans for disease resistance, adaptation to a wider range of environments, and early-maturity. We also hope to create bush-type popping beans.

Materials and methods: Fifty-eight genotypes from Peru, the CIAT germplasm bank, and the Core Collection were evaluated for popping ability in hot oil and in the microwave. The percentage of seeds that popped and the time to pop were measured (Table 32). In addition, the popping ability was rated on a 0-5 scale where 0 = no popping, 1 = seed coat splits and less than 10% expansion, 2 = up to 30% expansion, 3 = up to 50% expansion, 4 = up to 75% expansion, and 5 = 100% expansion. The best popping beans were selected and crossed to non-popping beans that were sources of BCMV and anthracnose resistance, early maturity, and bush bean architecture. We gave extra weight to crosses with popping beans that had type III (bush) growth habit because we wanted to develop bush-type ñuñas.

Results and discussion: Nine popping beans (six climbing and three bush types) were selected and crossed to the anthracnose resistance source (G 2333) and to three bush type non-popping beans (AFR 612, CAL 96, and CAL 143). The bush-type popping beans were G 11785, a small, round white-seeded genotype, G 23604, a large-seeded, black-speckled genotype, and G 23614, a brown-and-white-mottled genotype. Among the climbing bean popping beans were red- (G 12572), brown- (G 12621 and G 12623) and gray- (G 23691) seeded genotypes. The F_1s of simple crosses between bush-type popping bean and non-popping beans were double crossed amongst each other and backcrossed to the non-popping bean parent. Segregants from the white-seeded parent may be the most readily acceptable. Other combinations are being made among growth habits, seed colors, and popping ability.

Conclusions and future plans: How ñuñas pop is not well understood. We will be studying the inheritance of popping ability and developing markers to help expedite the transfer of the trait from ñuñas into bush types. Triple crosses will be made to incorporate anthracnose and BCMV resistance simultaneously into bush- and climbing-type popping beans. A wider range of anthracnose resistance sources will be used in the future. We will also use crosses to Gloriabamba, a Mesoamerican climbing bean variety released in Peru. With these crosses we hope to create new varieties of ñuñas that will increase the availability of this product. Increased supply might stimulate exports and marketing of popping beans from Peru. This project will work with the Peruvian Institute for Grain Legumes - IPEL (Instituto Peruano de Leguminosas de Grano) and the National University of Cajamarca to develop, test, and promote new popping beans.

Contributor: MW Blair

Table 32. Popping beans examined for popping ability in hot oil and in the microwave.

Genotype	Seeds popped (of 6)		Time to pop (minutes)		Popping scale (0-5)[a]	
	Microwave	Hot oil	Microwave	Hot oil	Microwave	Hot oil
Ñuña Pava	6	6	1.02	0.53	5	2
Ñuña Jabona	6	5	1.04	0.45	5	1
Mani Bola 12582	6	6	0.57	0.51	5	2
Ñuña Limona	5	6	1.12	0.50	4	1
Mani Palida	6	6	1.33	1.25	4	5
Huevo de Huanchaco	5	6	0.43	0.50	1	3
Ñuña Mani Roja	6	6	1.14	0.35	5	4
Ñuña Parcoyana	5	6	0.45	0.35	5	5
Ñuña Poroto Blanco	4	5	0.45	0.29	4	3
Ñuña Conejita	4	6	1.38	1.05	5	3
Ñuña Paloma	5	6	0.45	0.32	4	3
Ñuña Azul o Negra	6	6	1.13	1.00	1	0
Ñuña Poroto Canario	5	6	1.05	0.35	5	3
Ñuña Ploma	6	6	0.42	0.28	5	4
Ñuña Angel Poroto	4	6	0.40	0.30	4	4
G 11785	6	6	1.13	0.33	5	5
G 11786	6	6	1.00	0.39	5	3
G 11808	4	6	1.00	0.44	5	3
G 12572	6	6	0.58	0.39	5	5
G 12575	6	6	0.55	0.51	4	3
G 12588	6	6	1.17	0.54	4	5
G 12589	3	6	1.03	0.39	1	2
G 12621	6	6	0.50	0.39	5	5
G 12623	6	6	0.54	0.40	5	4
G 12627	3	5	0.48	0.42	3	4
G 12628	6	6	1.20	0.40	4	3
G 23603	1	5	1.19	0.32	0	4
G 23604	3	4	0.55	0.15	1	2
G 23604A	5	5	1.08	0.29	3	2
G 23605	2	6	0.53	0.41	5	5
G 23610	2	4	0.46	0.38	0	2
G 23612	4	3	1.40	0.32	2	3
G 23614	5	6	1.00	0.52	5	5
G 23643	5	4	0.54	0.33	3	2
G 23688	5	5	1.16	0.49	2	1
G 23691	6	6	1.05	0.45	5	4
G 23695	6	6	0.37	0.27	1	2
G 23697A	3	6	0.36	0.36	0	3
G 23701	4	6	0.58	0.43	4	3
G 23703	5	5	1.40	0.42	2	2
G 23704	4	6	0.40	0.32	0	2
G 23705	5	6	1.01	0.40	2	2
G 23706	5	6	1.02	0.39	5	4
G 23707	6	6	0.41	0.27	4	4
G 23710	6	6	0.55	0.40	2	3
G 23711C	4	6	0.50	0.39	1	3
G 23714	3	4	0.53	0.41	1	1
G 23716	5	5	0.54	0.48	1	2
G 23717	5	6	0.50	0.41	2	2
G 23719	6	6	1.26	0.43	5	3
G 23746	4	6	1.00	0.40	5	5
G 23754	6	6	0.48	0.32	4	2
G 23766A	6	6	0.46	0.34	5	4
G 23766B	5	5	0.45	0.35	1	1
G 23767	3	5	1.02	0.36	5	3
G 23767A	6	6	0.45	0.41	5	5
G 23874	3	6	0.58	0.31	0	1

a. where 0 = no popping, 1 = seed coat splits and less than 10% expansion, 2 = up to 30% expansion, 3 = up to 50% expansion, 4 = up to 75% expansion, and 5 = 100% expansion.

2.1.7 International Bean Nursery (IBN 2000); testing for angular leaf spot and common bacterial blight resistance

Rationale: Angular leaf spot and CBB are important foliar and seed-borne diseases of Andean beans grown in Africa and the Caribbean. We were interested in screening a nursery of Andean advanced lines for resistance to either or both diseases.

Materials and methods: The IBN 2000 nursery contained 40 Andean bush bean breeding lines from the AFR, AND, CAL, DFA, FOT, and POA series developed by J Kornegay. The lines included dark red kidney, radical, Sugar, and Canario grain types but most of the lines were red-mottled. These lines were tested for (1) yield potential in both Darien and Palmira, (2) resistance to Andean isolates of the ALS pathogen, *Phaeoisariopsis griseola*, in Darien, and (3) resistance to the bacterial blight pathogen, *Xanthomonas campestris* pv. *phaseoli* in Palmira. Randomized complete block designs (RCBDs) were used with three repetitions for each experiment. The check varieties A 483, AFR 188, AFR 612, AFR 619, A 36, CAL 143, Calima, Chocho, K 20, PC 50, PVA 773, Quimbaya, and Radical Cerinza were included.

Results and discussion: Good levels of resistance were found to the Andean isolates of ALS (Figure 42). Twenty-six lines had better resistance (score of 1 to 3) than any of the check varieties. The check varieties had ALS scores that ranged from 7 for K 20 to 3.5 for PC 50. Most of the lines were susceptible to CBB. Only six DFA lines had moderate resistance to this disease. The correlation between yield under disease inoculation and disease intensity was -0.765 for ALS and -0.363 for CBB. Yields in the disease inoculation trials were not correlated with yield potential in the uninoculated trials in Darien and Palmira. Therefore, both diseases had strong effects on the yield potential of these genotypes.

Conclusions and future plans: The current level of CBB resistance in Andean genotypes is insufficient and new emphasis will be given to this in the breeding program. A program to introgress CBB resistance from Mesoamerican sources such as VAX 6 is underway. Although ALS resistance exists in the advanced lines, it will be important to test the lines against Mesoamerican and Afro-Andean isolates.

Contributors: MW Blair, G Mahuku

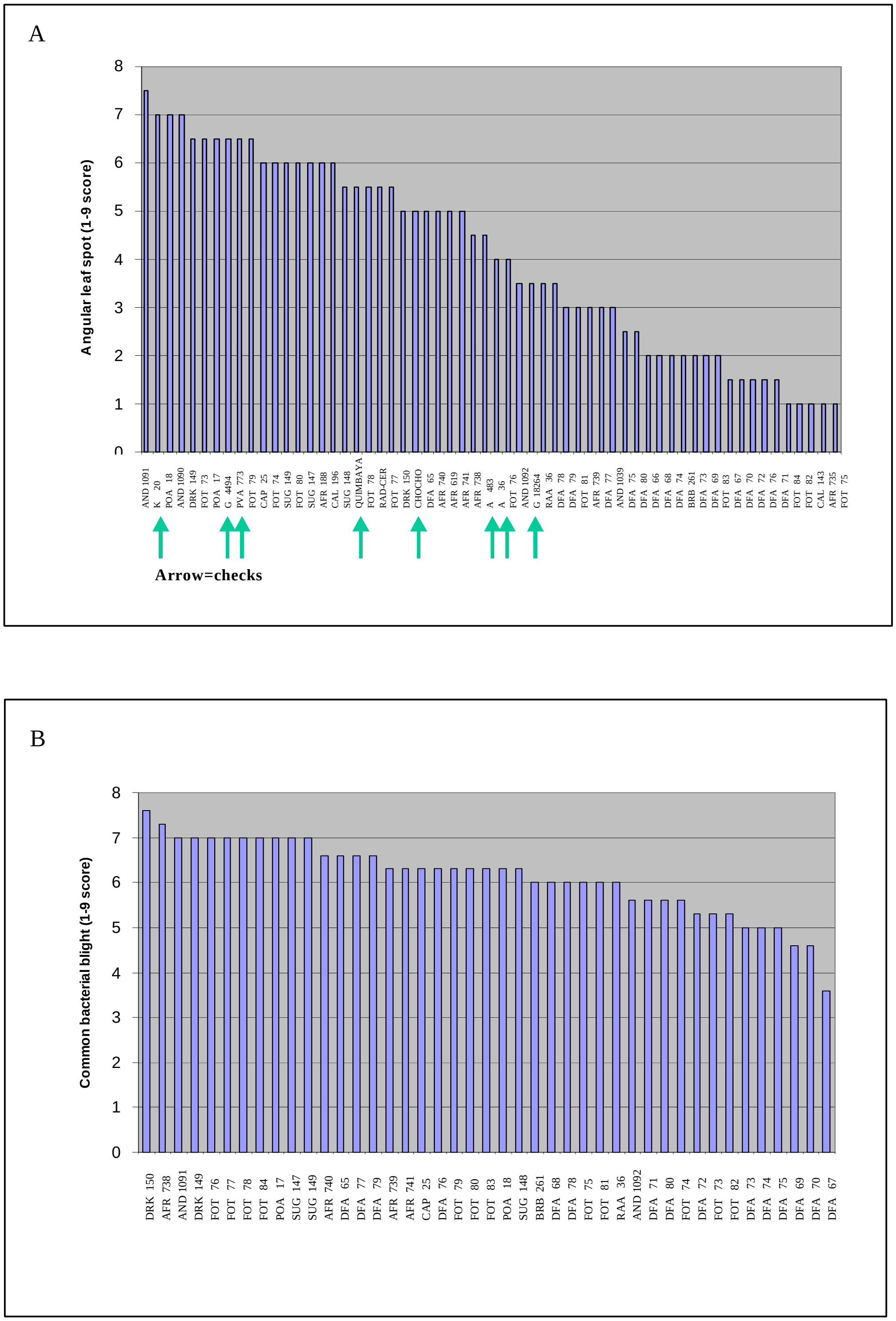

Figure 42. Disease reactions (1-9 scale) of genotypes from the IBN 2000 nursery with (A) Andean isolates of angular leaf spot in Darien 1999B, and (B) common bacterial blight in Palmira 2000A.

Progress towards achieving output milestones:

- **Lines combining resistance to BGMV, CBB, and BCMV distributed to the Caribbean and Andean Zone**

Red-mottled beans are a popular seed class in the Caribbean, Andean region, and Africa. We have developed multiple-disease resistant, red-mottled genotypes with a range of seed size and red color tones that contain combinations of resistance genes for BGMV, BCMV, and ALS. Marker-assisted selection was implemented to facilitate the breeding of BGMV resistance and will be used in the future for other diseases and insect pests. Work will continue on transferring the high level of resistance to CBB found in the VAX lines to red-mottled beans. *Empoasca* and bruchid resistances are being incorporated into the seed class as well. The red-mottled beans are being used to improve other Andean seed classes important to the region.

Activity 2.2 Developing germplasm resistant to insects

2.2.1 Pyramiding *Empoasca* resistance in red-mottled beans

See Activity 1.3, Section 1.3.3.

Activity 2.3 Incorporating wider genetic diversity into beans

Highlights:

- Higher yielding lines with commercial seed type were obtained from advanced backcross populations derived from crosses between cultivated Andean and wild common beans.

- Triple-cross Andean populations were developed to introgress traits from Mesoamerican climbing beans.

- The inheritance of low-phosphorous tolerance was studied in an Andean population.

- Andean climbing beans of heat-tolerant commercial type were developed and tested.

2.3.1 Analysis of Andean advanced backcross populations for yield traits derived from wild *P. vulgaris*

Rationale: The genetic diversity of cultivated *P. vulgaris* is thought to be narrower than that of wild common bean because of a genetic bottleneck that occurred during domestication. Yield-increasing alleles may still reside untapped in the wild accessions that could be exploited to improve cultivated beans. The advanced backcross method has been shown to be useful for incorporating wild germplasm into cultivar breeding programs for tomato and rice. Although wild beans have been used before to transfer resistance to diseases and insects, as in the noted case of the Arcelin gene that provides resistance to bruchids, the studies presented here are among the first to attempt to obtain a higher yield potential from wild beans. The objective of this research was to conduct a molecular analysis of two advanced backcross populations to find QTL derived from wild beans that can be useful in the improvement of cultivated beans.

Materials and methods: Four Andean advanced backcross populations were planted over eight seasons. The populations were derived from two backcrosses of wild beans to a Colombian large red-seeded "Radical" type, commercial variety, ICA Cerinza. The wild donor parents were G 24390 (from Mexico), G 24404 (Colombia), G 10022 (Mexico), and G 23585 (Peru). Each population consisted of 95 selected lines. Some of the populations also had from 62 to 215 additional lines. The selected lines were yield-tested in three locations for each of the G 24390 and G 24404 populations; and in one location for the G 10022 and G 23585 populations. The field sites included Isabela (Puerto Rico), Palmira (Valle, Colombia), Popayán (Cauca, Colombia), and Darien (Valle, Colombia). Three repetitions in a lattice design were used for each of the experiments, except in Puerto Rico where an RCBD with two repetitions was used. The check varieties were AFR 612, Rosada, or Quimbaya, depending on the environment. Two of the populations were genotyped with molecular markers and analyzed for QTL as described in the SB-2 Annual Report.

Results and discussion: In all four advanced backcross populations some progeny significantly out-yielded Cerinza (Table 33), which had an average yield across the eight environments of 1162 kg ha^{-1}. Meanwhile, the yield increase of the best line over the recurrent parent averaged 61.7% (or 407 kg ha^{-1}) across the eight experiments. The yield of the best line even surpassed the local check in four of the eight environments. In each experiment, Cerinza yielded more than the average yield of all $BC_2F_{3:5}$ progeny of the population, indicating that the progeny still contained negative factors from the wild that depress yield compared to the recurrent parent.

Table 33. Yield data (kg ha^{-1}) for four Andean advanced backcross populations derived from Cerinza as recurrent parent and four wild beans, G 24390, G 24404, G 10022, and G 23585 as donor parents.

	G 24390			G 24404			G 10022	G 23585
	Popayán 1998B	Puerto Rico 1999A	Palmira 1999B	Popayán 1998B	Popayán 1999A	Darien 1999B	Palmira 1999B	Darien 2000A
Best line	1374	2067	592	1139	509	2114	1840	2917
Average 10 best	1190	1864	542	1086	439	1759	1698	2513
Cerinza	915	1680	434	759	146	1747	1367	2250
Local check	1172	2347	703	1258	340	1905	2216	2719
Variety used	AFR 612	Rosada	Quimbaya	AFR 612	AFR 612	AFR 612	Quimbaya	AFR 612
Average $BC_2F_{3:5}$	845	1407	377	813	236	1333	1300	1904
Number of reps	3	2	3	3	2	3	3	3
CV	33.2	31.7	32.7	33.9	47.9	30.51	15.8	23.3
LSD (P = 0.05)	337	905	186	418	158	563	332	654
Increase/ Cerinza	459	387	158	380	363	367	473	667
Increase (%)	50.2	23.0	36.4	50.1	248.6	21.0	34.6	29.6

Quantitative trait loci analysis supports the observation that some introgressions from the wild parent have a detrimental effect on yield. Significant QTL for yield were found in both the Cerinza x G 24390 and Cerinza x G 24404 populations. However, most of the QTL associated with the wild alleles were negative, in effect positive QTL whose source was Cerinza. This indicates that a large number of the alleles were still transmitted from the wild parent that had negative effects on yield and that remained to be eliminated from the progeny. Positive QTL were found for the two populations analyzed for markers when grown in Popayán. This field site at 2100 m was closer to the ideal growing environment for the Cerinza variety and therefore a more appropriate testing site for advanced backcross progeny derived from Cerinza. In Popayán, the positive QTL had phenotypic effects that ranged from 99 to 225 kg ha^{-1} increased yield and these significant QTL explained from 7% to 22% of the variance for yield in these seasons. This suggests that MAS of QTLs could be successful at transferring these yield genes into other commercial seed-types.

Future plans: Two of the populations are being analyzed in collaboration with CORPOICA in Nariño. These studies will be definitive because they are being conducted in regions where ICA-Cerinza is a popular commercial variety among farmers. The QTL identified in this study are being introgressed into other Andean seed types through crosses between the F_1 plants of the first backcross of G 24390 x Cerinza and commercial varieties. These include varieties such as CAL 96, a red-mottled variety released in Uganda and Malawi, ICA Quimbaya, a red kidney variety released in Colombia, and PC 50, a Pompadour variety released in the Dominican Republic. The QTL analysis of some of these populations is described in more detail in the SB-2 Annual Report. We will compare the location of QTL found for the Andean populations. We will also analyze the QTL controlling seed size, days to flowering, and days to harvest to determine if these are associated with the yield QTL identified here.

Contributors: MW Blair, S Beebe, A Hoyos, G Iriarte

2.3.2 Use of triple-cross Andean populations for the introgression of traits from the Mesoamerican climbing beans

Rationale: Populations developed from Andean by Mesoamerican bush bean gene pools frequently have been unproductive in terms of developing new bean varieties. The reason for the poor phenotypes of inter-gene pool hybrids may be the complex epistatic interactions that occur in progeny derived from such wide crosses. Backcrossing has been used to overcome this problem and to introgress specific traits from one gene pool to the other. We were interested in trying a different approach by making inter-gene pool crosses between Andean bush and Mesoamerican climbing beans and triple crosses between their hybrid and another Andean bush bean parent. We hoped to answer the following questions. Is one backcross to the Andean bush habit enough when incorporating genes from Mesoamerican climbing beans? Can the heterosis that has often been seen in crosses between Andean bush by Mesoamerican climbing beans be fixed to produce a yield advantage in Andean bush bean varieties?

Materials and methods: Fifteen simple crosses and 100 triple crosses were made. The Mesoamerican climbing bean parents were chosen based on their resistance to ALS, anthracnose, bean fly, or low phosphorous. The Andean bush beans used as parents in the simple cross provided resistances to BCMV and drought. The final parents in the triple crosses were also Andean bush beans from various commercial seed classes. These included red-mottled (AFR 619, AFR 699, BRB 198, CAL 96, CAL 143, G 13910, Paragachi, POA 12), large red (DRK 57, Montcalm), purple-speckled (G 15430), and cream-mottled (SUG 131, SUG 137) beans. Triple-cross F_1 plants were bulk harvested in Darien in 1999B. In the following season, both simple- and triple-cross F_2 populations were grown in Darien both under trellises and in field rows and in Palmira under trellises. Single plant selections were made for both bush and climbing growth habits.

Results and discussion: In total, 2310 selections (1662 bush and 648 climbing beans) were made in the simple- and triple-cross populations. Across both environments, 57% of the selections out of the simple crosses were climbing beans, while only 24% of the selections out of the triple crosses were climbing beans (Table 34). More climbing bean selections were made for the triple crosses in Palmira than in Darien. This difference may have been because of the interaction of the climbing bean phenotype with temperature and fertility, which are higher in Palmira, along with the trellising for support. Indeed many of the climbing bean selection were determinate, but reached a height of 2 meters in Palmira. Meanwhile, in Darien, the triple crosses planted under trellises produced few (11%) climbing bean selections.

Table 34. Single plant selections (SPS) from simple and triple crosses involving Mesoamerican climbing beans and Andean bush beans.

Location / population	Bush SPS	Climbing SPS	Climbing (%)
Palmira - trellis:			
Simple crosses	27	86	76
Triple crosses	136	324	70
Darien:			
Simple crosses	97	78	44
Triple crosses	1402	160	11
Across environments:			
Simple crosses	124	164	57
Triple crosses	1538	484	24
Total	1662	648	-

Conclusions and future plans: The F_3 families from the climbing and bush bean selections with the best seed type and plant architecture will be advanced in either Darien or Palmira. Heat-tolerant Andean climbing beans will be selected in Palmira. Yielding ability of the bush bean selections will be measured. The selected families will be screened with disease inoculation to confirm the introgression of resistance genes from the Mesoamerican parents.

Contributors: MW Blair, S Beebe

2.3.3 Inheritance of low phosphorous tolerance in the Andean recombinant inbred line population AND 696 x G 19833

Rationale: Of the soils in Latin America, 82% suffer from phosphorous deficiency, which is widespread in East Africa as well. Studies at CIAT have identified several sources of tolerance to low-P conditions. These genotypes can take up or use phosphorous more efficiently. This study aimed to confirm that G 19833 is a source of tolerance to low-P stress and to compare the inheritance of tolerance in the AND 696 x G 19833 population of RILs to that in other Mesoamerican and inter-gene pool populations. We were also interested in comparing the phosphorous efficiency of segregants from this population with determinate versus indeterminate growth habit.

Materials and methods: The population was planted in Darien (Valle, Colombia) in March 2000 in a 9 x 9 lattice design with three repetitions under high- and low-P conditions. The Darien field site has very low natural levels of phosphorous in the soil (< 8 ppm). The low-P treatment in the experiment received 30 kg ha^{-1} while the high-P treatment received 300 kg ha^{-1} of superphosphate fertilizer. Seventy-seven RILs were planted plus four check varieties, G 19833, G 4017 (Carioca), and G 16140. Data were collected on growth habit, days to flowering, days to harvest, and yield per plant and per plot.

Results and discussion: The yields in the high-P treatment were 136% higher on the average than were those in the low-P treatment. Among the RILs, some were both responsive to P fertilization and adapted to low-P conditions (Figure 43). The correlation between yield in high- and low-P environments was $r = 0.584$. Among the check varieties, G 4017 was the highest yielding in both fertilizer treatments; G 16140 was the most efficient under low phosphorous. G 19833 was low yielding in high P and moderate yielding in low P. Of the 77 RILs tested in this trial, 56 had a determinate growth habit and 21 were indeterminate (Figure 44). In the low-P treatment, the average yield of indeterminate lines was higher than that of the determinate lines. The peak in the distribution of yields among the determinate RILs was higher than for the indeterminate lines. Meanwhile, in the high-P treatment, the average yield of the indeterminate lines was lower than that of the determinate lines. In the high-P treatment, the population distribution for yield of both the determinate and indeterminate lines was bimodal suggesting that a single gene was determining adaptation. Many of the RILs that were indeterminate were late and unadapted like the G 19833 parent. Even though G 19833 and the late RILs produced a large amount of biomass, their photoperiod sensitivity did not allow these genotypes to reach their full yield potential.

Conclusions and future plans: On low-P soils, the yield potential of the indeterminate genotypes in the population was higher than that of determinate genotypes. However, on high-P soils the indeterminate genotypes do not appear to provide a yield advantage and indeterminacy may be associated with a lack of reproductive adaptation associated with the G 19833 source of tolerance.

Contributors: MW Blair, S Beebe, A Hoyos

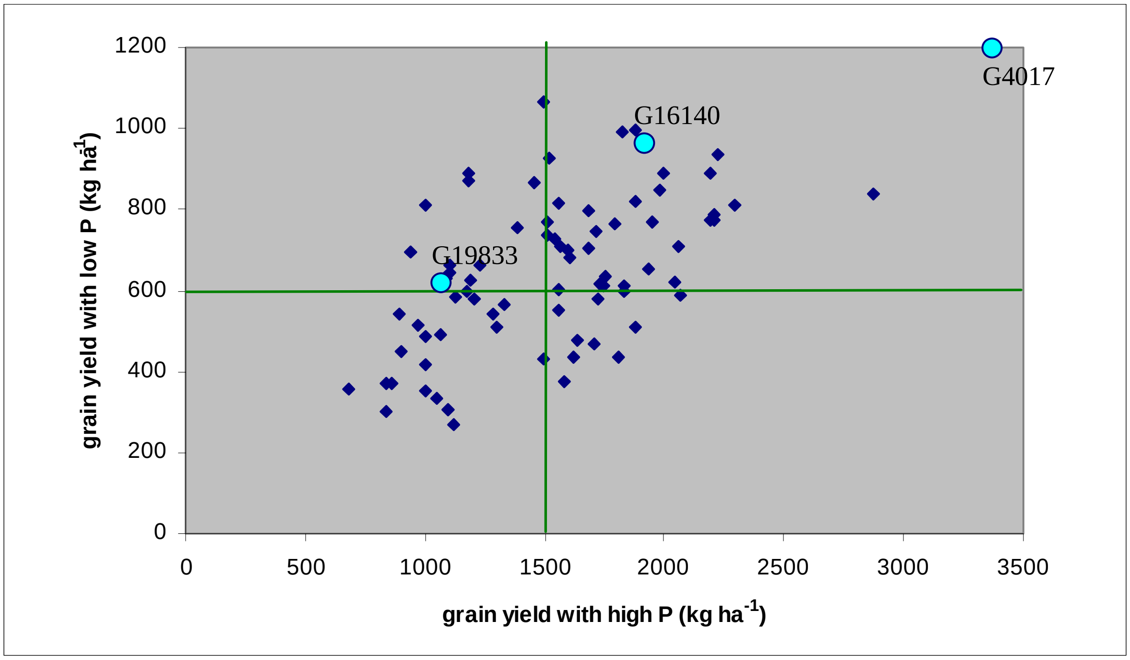

Figure 43. Regression of grain yield under low- and high-phosphorous treatment for recombinant inbred lines from the AND 696 x G 19833 population.

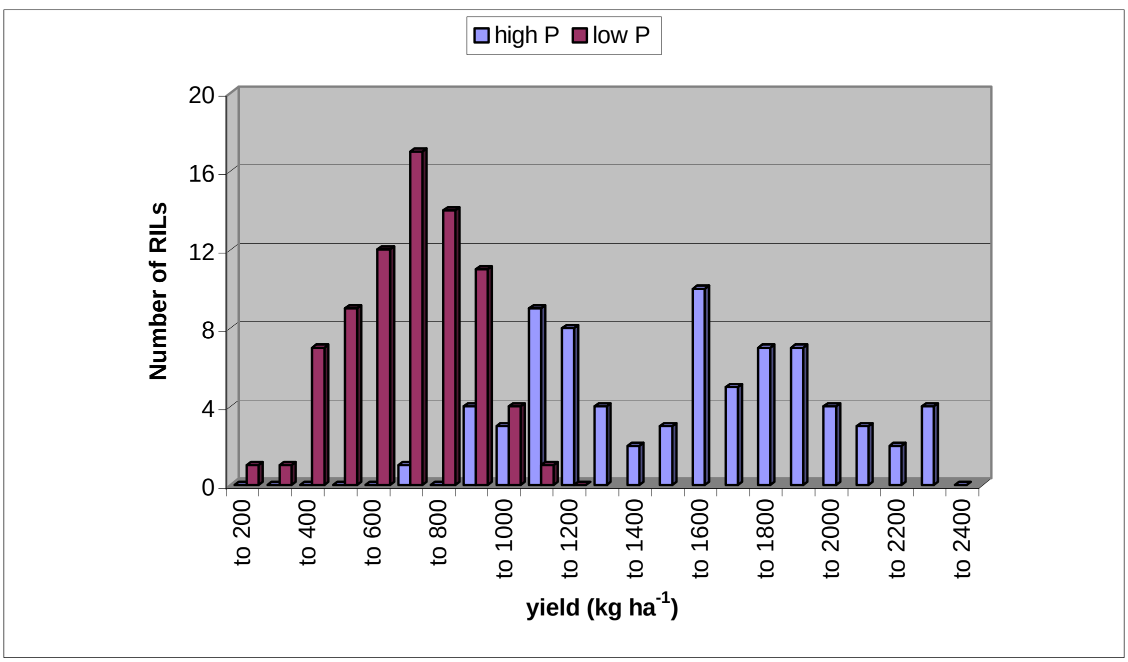

Figure 44. Population distribution of grain yield under high- and low-phosphorous treatment for recombinant inbred lines from the AND 696 x G 19833 population.

2.3.4 Development of mid-elevation, commercial-type, Andean climbing beans

Rationale: The most outstanding characteristic of climbing beans is their high yield potential compared to more commonly grown bush beans. Climbing beans have been an important component of traditional societies in Central America and the Andes for centuries. More recently, climbing beans have become important in certain areas of Africa. The principal limitation to the expansion of climbing bean technology into new areas has been the lack of new varieties. Most currently available climbing beans come from high-altitude areas of Central and South America and do not grow well in lower elevations or hotter climates. An urgent need exists for climbing bean varieties that are adapted to lower elevations (800 to 1800 m) and resistant to the diseases encountered there. Currently, very few climbing bean varieties are available with the red-mottled or red kidney seed types, which are preferred in many areas of the Caribbean, Africa, and South America. Therefore, an additional challenge for breeders is to develop climbing bean varieties that produce grain with the proper color and size. The objective of this research is to hybridize, develop, and test mid-altitude adapted, commercial-type, Andean climbing beans.

Materials and methods: In 1999B, we initiated screening for climbing beans with adaptation to Palmira (1000 m). We planted four sets of germplasm, including 61 accessions from the Core Collection, 92 accessions from Mexico, 182 accessions from Rwanda, and 50 bulk selections made from Andean climber x Andean bush crosses. Single plant selections were made from the most promising bulks. In the second season in 2000A we repeated some of the germplasm and also planted out F_2 seed from 15 simples crosses between Mesoamerican climbers and Andean bush beans, as well as from 24 triple crosses between Andean bush x (Mesoamerican climber x Andean bush beans). The climbing beans were all planted at a low density of 10 plants per meter of linear row with 1.2 m between rows and the vines were supported on bamboo and wire trellises at about 2.0 m aboveground.

Results and discussion: The accessions from the Core Collection adapted to the Palmira site were almost entirely Mesoamerican genotypes and therefore were related to the accessions tested from Mexico. The Rwandan accessions were highly diverse in terms of seed color and their climbing ability and probably represent natural hybridizations between climbing and bush beans from both Andean and Mesoamerican gene pools. The best red-seeded types from Rwanda and Mexico were included in a larger trial the following season. Single plant selections were made in the $F_{5:7}$ generation of Andean climber x Andean bush populations based on the best seed types in the bulk. The F_7 lines were then tested in the second season to confirm their adaptation to mid-elevation regions and increase seed. Of the selections, 27 were red-mottled, 13 were large red, and 32 were cream-mottled, all with average seed sizes around 50 g per 100 seed. Figure 45 shows the yield of these genotypes. The new Andean climbing bean lines yielded much better than unadapted highland climbers such as ICA Viboral and LAS 399 (Andeans) and G 2337 (Mesoamerican). The yield advantage of the adapted climbing beans over bush beans, such as Kirundo, AND 930, Rojo 70, MAM 39, DRK 49, and SUG 92, was evident. This is even more significant because these were the bush bean parents of the advanced lines. Some of the new Andean climbing beans yielded as well as the Mesoamerican climbing beans, G 2333, G 685, and Magdalena 3, which are all standard varieties throughout climbing-bean growing areas of Africa.

Conclusions and future plans: We are hybridizing best accessions from Mexico, Rwanda, and the Core Collection with single plant selections from Andean populations with tolerance to higher temperatures. We are also beginning to pyramid in the resistance and tolerance factors (ALS, BCMV, BGMV, CBB, bean fly, bruchid, *Empoasca*, and low fertility) that will be necessary for successful production of climbing beans at mid-elevation, bean-producing areas. New lines developed from these crosses will be included in an international climbing bean nursery to test the best advanced lines and accessions at a range of elevations. We hope to test early generation climbing bean populations in participatory plant breeding projects and on-farm trials.

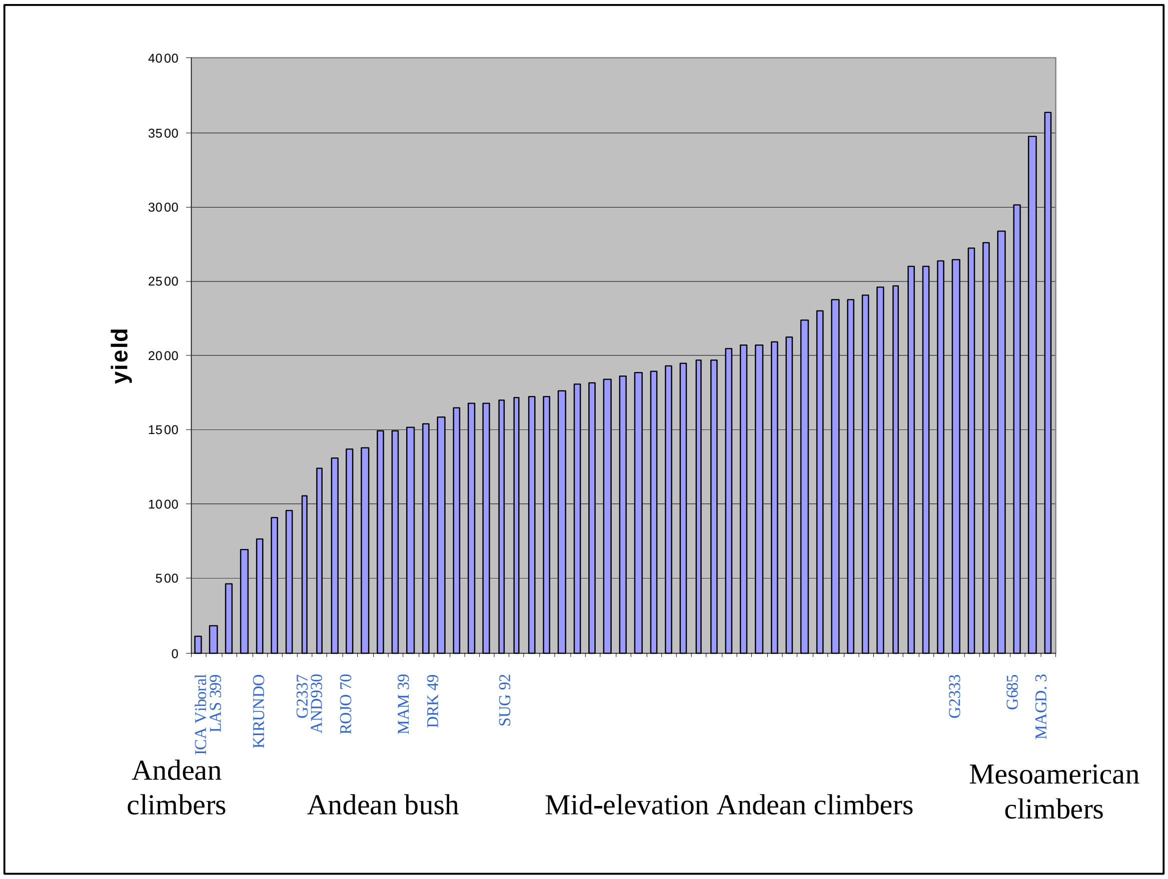

Figure 45. The yield (kg ha^{-1}) of mid-elevation, heat-tolerant, Andean climbers compared to Andean bush beans, Mesoamerican climbing beans, and unadapted Andean climbing beans.

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Progress towards achieving output milestones:

- **Phosphorous efficient genotypes developed**

The inheritance of low-phosphorous tolerance from G 19833 was studied in an Andean background. Interactions with determinate growth habit will be studied further.